

**U.S.S.N. 09/601,645**  
**DAHM *et al.***  
**AMENDMENT**

72. A method for the quantification of tumor cells in a body fluid, comprising:

(a) concentrating tumor cells in a sample of a body fluid by treating the sample of body fluid with a cell separation medium;

(b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and

(c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid.

73. The method of Claim 71, further comprising prior to amplification, preparing cDNA from the mRNA contained in the sample.

74. The method of Claim 71, wherein one or both of the following oligonucleotide primers are used for the amplification:

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGGA AAGC 3' SEQ ID No. 1,

designated TRT1; and

5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' SEQ ID No.2, designated TRT2,

wherein each of hTRT1 and hTRT2 optionally further comprises a promoter sequence for an RNA polymerase.

75. The method of Claim 71, wherein step (a) comprises:

(i) covering a cell separation medium with a layer of the body fluid;

(ii) centrifuging the cell separation medium covered with the body fluid;

and (iii) collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid.

76. The method of Claim 71, wherein the tumor cells are selected from cells of metastasizing tumors and/or neoplasms, wherein the cells are derived from tumors and cells selected from the group consisting of a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma,

**U.S.S.N. 09/601,645**  
**DAHM *et al.***  
**AMENDMENT**

neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma and lymphoma cells.

77. The method of Claim 75, wherein the cell separation medium has a density in the range of from 1.055 to < 1.070 g/ml.

78. The method of Claim 71, wherein the sample is blood, and the tumor cells from the blood sample are concentrated.

79. The method of Claim 72, further comprising prior to amplification, preparing cDNA from the mRNA contained in the sample.

80. The method of Claim 72, wherein one or both of the following oligonucleotide primers are used for the amplification:

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGGA AAGC 3' SEQ ID No. 1, designated TRT1; and

5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' SEQ ID No.2, designated TRT2,

wherein each of hTRT1 and hTRT2 optionally further comprises a promoter sequence for an RNA polymerase.

81. The method of Claim 72, wherein the tumor cells are selected from cells of metastasizing tumors and/or neoplasms, wherein the cells are derived from tumors and cells selected from the group consisting of a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma, neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma and lymphoma cells.

82. The method of Claim 72, wherein the cell separation medium has a density in the range of from 1.055 to < 1.070 g/ml.

83. The method of Claim 72, wherein the tumor cells are separated from telomerase-positive non tumor cells.

84. The method of Claim 72, wherein the sample is blood, and the tumor cells from the blood sample are concentrated.

85. The method of Claim 71, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

86. The method of Claim 72, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

Please replace claims 1, 21-24, 26-30, 34, 61, 62, 64, 69 and 70 with amended claims 1, 21-24, 26-30, 34, 61, 62, 64, 69 and 70 as follows:

1. (Amended three times) A method for the quantification of tumor cells in a body fluid, comprising:

(a) concentrating tumor cells in a sample of a body fluid by covering a cell separation medium with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid;

(b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; and

(c) quantitatively determining the amount of amplified nucleic acid, thereby quantifying tumor cells in a body fluid.

21. (Amended twice) The method of Claim 1, wherein the cell separation medium has a density in the range of from 1.060-1.067 g/ml.

22. (Amended twice) The method of Claim 1, wherein the centrifugation is carried out at about 1000 x g for about 30 minutes.

23. (Amended twice) The method of Claim 1, wherein the cell separation medium used is Percoll or Ficoll.

24. (Amended twice) The method of Claim 1, wherein the body fluid is blood and prior to applying the body fluid sample to the cell separation medium,

**U.S.S.N. 09/601,645**  
**DAHM *et al.***  
**AMENDMENT**

the body fluid is mixed with one or more substances that prevent aggregation of platelets to tumor cells, and/or prior to applying the body fluid sample to the cell separation medium, the body fluid is freed of substances that promote aggregation of platelets to tumor cells.

26. (Amended twice) The method of Claim 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent.

27. (Amended twice) The method of Claim 11, wherein the peripheral blood is venous or arterial blood.

28. (Amended twice) The method of Claim 1, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

29. (Amended three times) The method of Claim 1, wherein after centrifugation and before collecting the tumor-cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers.

30. (Amended twice) The method of Claim 1, wherein the centrifugation is carried out in a vessel that is divided by a porous barrier, a filter or a sieve into an upper and a lower compartment and the body fluid is introduced into the upper compartment.

34. (Amended three times) The method of Claim 1, wherein a dye is added to color the cell separation medium, whereby the color of the cell separation medium is distinguishable from that of the supernatant body fluid.

61. (Amended) The method of Claim 78, wherein concentration is effected by immunoabsorption.

62. (Amended) The method of Claim 1, wherein the cell separation medium has a density in the range of from 1.055 to < 1.070 g/ml.